NON-HLA GENE POLYMORPHISM IN PULMONARY TUBERCULOSIS

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INTRODUCTION

BCG vaccination has been shown to give protection against tuberculosis. However, South Indian (Chingleput) Trial of BCG vaccination did not give any protection against bacillary forms of tuberculosis. A number of hypotheses and possibilities were put forward for this failure (1). One of the possibilities suggested was the genetics of the people (Host genetics) living in that region. Pulmonary tuberculosis is a granulomatous lung disease caused by Mycobactrium tuberculosis. Susceptibility to tuberculosis has been suggested to be multifactorial. Though environmental and socio-economic factors are primarily related, numerous studies have emphasised the importance of host resistance and hereditary susceptibility (2,3).

The main objective of this project [**Part of the Indo-UK DFID (Department For International Development)** funded project] was to investigate the host molecular genetics in order to understand the failure of BCG vaccination as well as better management of tuberculosis.

The host genetics factors may be divided into HLA (Human Leucocyte Antigen) and Non-HLA genes.

HLA-studies in pulmonary - TB

HLA-studies carried out in Indian, Indonesian and Russian pulmonary tuberculosis patients revealed the HLA-DR2 association with pulmonary tuberculosis. Our recent study (4) also confirms HLA-DR2 association with PTB. Though HLA-DR2 association with PTB is significant ($P<0.001$; Relative Risk 2.3) (Table) it is only a minor association. which cannot be used as a genetic marker to predispose the disease, (e.g. HLA-B27 is being used as a HLA-genetic marker for ankylosing spondilytis). This suggests that association of Non-HLA genes with pulmonary tuberculosis may also be possible. Recently, association of multicandidate genes including HLA and Non-HLA genes have been suggested for various infectious disease (5).

Non-HLA gene polymorphism

Human genome analysis revealed several candidate Non-HLA genes. Due to point mutations, most of these Non-HLA genes occur as diallelic polymorphic forms. Such polymorphic genes have been shown to be associated with the susceptibility to a number of infectious and noninfectious diseases (5).

To find out whether Non-HLA genes are associated in the susceptibility to pulmonary tuberculosis, the following Non-HLA gene polymorphisms, were studied in pulmonary tuberculosis patients (n=202) and control subjects (n=109).

1. Mannose Binding Protein (MBP) genes

Mannose binding protein plays an important role in the host defence against pathogens. Mutations in the genes of mannose binding protein results in low plasma level of this protein which leads to susceptibility to infection. In the present study, wild type and mutant allets of MBP 52, 54 and 57 region of the genes were studied by amplifying the MBP genes of the genomic DNA and the PCR product (bp339) was dot-blotted on nytran membrane and probed with specific oligonucleotide probes and detected using non-radioactive chemiluminiscent system.

2. IL-1 Receptor Antagonist (IL-1RA) gene

IL-IRA is a cytokine which competes for IL-1 binding site and regulates the production of IL-1. 86 base pair tandem repeat mini satellite polymorphism have been identified. This polymorphisms was studied using PCR product and size variants were typed on a 2% agarose gel. Based on the number of 86 bp copies six alleles have been assigned. The percent genotype frequency of these alleles have been studied.

3. Vitamin-D Receptor (VDR) gene

Vitamin-D3 (1,25 dihydroxy vitamin-D3) regulates calcium metabolism. It is an immunoregulatory hormone and activates monocytes and stimulates cell-mediated immune response. The effects are exerted by interaction with the Vitamin-D receptor which is present on monocytcs and activated T & B lymphocytes. VDR is a nuclear hormone receptor. Point mutation in the VDR region results in reduced mRNA expression. Single base change polymerphisms of VDR gene were studied.

VDR region of the genomic DNA was amplified, a 361 bp PCR product was dot blotted and detected using specific oligonucleotide probes and chemiluminiscent system.

4. Tumor Necrosis Factor Alpha Gene (TNF a)

Tumor necrosis factor alpha is an inflammatory cytokine mainly produced by monocytes and macrophage. This cytokine plays an important role in pathogenesis of severe infectious disease. Promoter region polymorphism (G-> A mutation at - 308)

affects the regulation of transcriptional start site of TNR alpha gene. Two alletic forms TNF1 (wild) and TNF2 (mutant) have been assigned. TNF2 allele is associate with higher constitutive and inducible levels of transcription than the TNF1 allete. Similarly, another gene polymorphic at 238 promotor region (G-> A mutation) has been identified. For the present study both - 308 and - 238 TNF alpha polymorphisms have been studied.

TNF alpha region of the genome was amplified (PCR product size 693 bp) and polymorphism at - 308 and - 238 have been studied using dot-blot, oligonucleotide probes and chemiluminiscent detection system.

5. ***Inducible Nitric Oxide Synthase (iNOS) gene**

Nitric oxide has been shown to be microbicidal. Inducible nitric oxide synthase (iNOS) is transcriptionally regulated enzyme that synthesises nitric oxide from L-arginine that has a key role in the pathophysiology of systemic inflammation. CA repeat microsatellite polymorphism has been shown at 313 and 317 region of the iNOS gene. The iNOS gene region was amplified (PCR product : 317 bp) using specific primers with fluorescent lables. CA repeat microsatelite polymorphism was studied by gene scan analysis.

6. *Natural Resistance associated macrophage protein-1(NRAMP-1)

In mice BCG gene has been shown to affect resistance to several intracellular pathogens such as Leishmania parasites (Lsh). Salmonell (Italy) and some strains of Bacille Calmette Guerin (BCG). The equivalence for the BCG gene is the NRAMP-1 gene. The gene encodes for the natural resistance associated macrophage protein (NRAMP) which is involved in macrophage activation. This gene is identified as a candidate for the murine macrophage resistance gene against intracellular pathogens. The human homologue of NRAMP- 1 has, CA repeat microsatellite polymorphisms at 199 and 201 region of the human NRAMPM-1 gene. The NRAMP-1 gene was amplified using specific primers with fluorescent labels. The CA repeat polymorphism was studied by gene scan analysis.

*Both iNOS and NRAMP-1 gene polymorphisms were done in collaboration with Richard Bellamy, WTCHG, Oxford, UK.

Results

Genotyping of MPB 52, 54 and 57 variants showed a significant increase of functional mutant homozygotes of the over all MBP genes in PTB patients than control subjects (P=0.008) (Table).

TABLE
PERCENT PHENOTYPE FREQUENCY OF HLA-DR2 AND GENOTYPE FREQUENCY OF FUNCTIONAL MUTANT HOMOZYGOTES OF MANNOSE BINDING PROTEIN GENES OF CONTROL SUBJECTS AND PULMONARY - TB PATIENTS

Candidate Genes	Control Subjects	PTB Patients	P Value	Relative risk/Odds ratio
HLA* HLAW-DR2	29.5 (n-122)	48.8 (n=209)	P<0.001	2.3 RR
Non-HLA* MBP-FMH	1.8 (n= 109)	10.9 (n=202)	P=0.008	6.5 OR

Results taken from Ref. No.4 and Ref. No.7 respectively.

MBP - FMH : Functional Mutant Homozygotes of Mannose Binding Protein genes.

Numbers in the parentheses represent the actual number of control subjects and patients studied.

RR - Relative Risk : OR : Odds ratio.

None of the IL-1RA alleles was associated with pulmonary tuberculosis. However, allele six (single repeat-around 118 bp) was seen in one patient ourr of 202 patients (percent genotye frequency 0.5%) and not in control subjects. So far single repeat has not been reported in other populations.

No association was seen with other gene polymorphisms studied in pulmonary - TB patients and control subjects.

CONCLUSION

The functional mutant homozygotes of the overall mannose binding protein gene variants 52, 54 and 57 are associated with pulmonary tuberculosis.

IL-IRA, VDR, TNF-alpha, iNOS and NRAMP-1 genes are not associated with the susceptibility to pulmonary - TB. The present study suggests that Non-HLA genes are also associated in the susceptibility to PTB, apart from HLA from HLA-DR2.

Identification of new host-genes that are associated with the susceptibility and resistance to *M. tuberculosis* in infection/disease development, may be beneficial and a battery of susceptibility genes for TB may serve as genetic markets to predetermine the development of the disease.

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